

Progress report restraining ruminants

Upright versus inverted restraining

Marien Gerritzen, Henny Reimert, Joop van der Werf, Vincent Hindle, Kathalijne Visser en Ingrid van Dixhoorn

CONFIDENTIAL

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Preface

Arising from the debate concerning a covenant on slaughter without stunning, the Dutch minister of Economic Affairs has, in close compliance with concerned parties, listed areas requiring further investigation aiming at safe guarding or improving animal welfare during slaughter without stunning ("Agreement on unstunned slaughtering in accordance with religious rites", June 2012). These areas include:

- Identification of critical control points.
- Validation of parameters to examine unconsciousness
- Reduction in time between cutting and loss of consciousness
- Number of neck cuts required.
- **Method and duration of restraint**

In this report we present the results of a study into the method of restraining, more specific the level of inversion compared to upright restraining.
This work falls within the policy support research programme Animal Welfare (BO-20-08) of the Ministry of Economic Affairs. The report presents the results of a study on physiological and behavioural response in cattle to being restrained in an upright, sideways and inverted position.

Summary

At present, knowledge concerning the effect of current restraining methods on the welfare of cattle and small ruminants is limited; and especially the impact of positioning and rotating the animal warrants further study. In addition, information is scarce concerning the methods and numbers of cattle, sheep and goats that are being restrained and rotated in practice. As a response to parliamentary discussions, the Ministry of Economic Affairs has commissioned a study into the methods of restraint, with special emphasis being accorded to position of the animal (large and small ruminants) during restraint and rotation. The aim of the present study was to gain insight into the levels of stress animals encounter during different methods of restraining and rotating.

Impact on animal welfare of up-right versus inverted restraining was assessed based on behavioural parameters and physiological parameters. The experiment has been conducted at a test facility to facilitate standardisation of the procedure. The experiment has been designed for nine culled dairy cows. All nine animals have been exposed to three different treatments following a 3x3 latin square design. The treatments were: 1) Restraining in upright position, 2) Restraining followed by 90 degrees rotation, 3) Restraining followed by 180 degrees rotation (inverted position/dorsal recumbence). The rotation phase lasted a maximum of three minutes. The animals were allowed a week to recover between treatments. It was hypothesized that repeated exposure to the restraining pen may lead to either habituation or aversion. A randomized experimental design helped to detect the treatment impact on the animals and any habituation to the treatment. To measure physiological parameters all animals had a surgically implanted telemetry transmitter (Data Science International, DSI®, St Paul, MN) allowing registration of body temperature, activity, blood pressure and cardiac activity (ECG) during the experimental period. For behavioural parameters animals were equipped with a non-invasive sensor to detect ruminating, eating and drinking behaviour (RumiWatch® (ITIN+HOCH; Ettenhausen, CH)) and activity was recorded with 3-dimensional accelerometers, commonly referred to as pedometers, at 4 Hz (IceQube™, IceRobotics Ltd., South Queensferry, UK). Furthermore, behavioural observations during driving the animals towards the restraining pen were made. These included vocalisations, resistance to move, showing white of the eyes, posture, defecating, urinating, turning, and handling such as stimulation needed to move the animals from one area to the other area.

So far, results indicated a significant effect of previous experience in the restraining pen on time required and the amount of stimulation needed to drive the animals out of a starting pen towards the restraining pen ($F=5.5$, $p=0.022$ and $F=601$, $p<0.001$, respectively). Animals without previous experience in the restraining pen took less time and less encouragement was needed compared to animals with previous experience of being in the restraining pen. During the phase of the treatment in which the animals were rotated 0, 90 or 180 degrees the behavioural parameter showing white of the eyes differed significantly between treatments ($F=4.11$, $p=0.016$). Animals restrained and not rotated (0 degrees rotation) displayed less white of their eyes compared to animals that were rotated 90 or 180 degrees. Heart rate patterns over time differed significantly between treatments ($F=2.85$, $p<0.001$); whereas the heart rate of animals restrained without rotation resided between 88 and 95 beats per minutes, for rotated animals the heart rate decreased to about 76 to 80 beats per minute. While there was no effect of treatment on the pattern of diastolic blood pressure during the three minutes of treatment (rotation 0, 90, 180 degrees) there was a significant effect of earlier experience in the pen. Animals with prior experience in the restraining pen (in this experiment) showed a significant drop in diastolic blood pressure ($F= 2.66$ $p=0.047$) over the period of three minutes in the restraining pen, regardless the treatment. To study the effect of treatment on the recovery, laying activity and rumination activity were monitored. There was no effect of treatment on either latency to start laying or ruminating nor the length of the first laying or rumination bout.

Summarizing, the results of the current study show that restraining and rotation is stressful for cows. It has been shown that there is an acute stress effect on being rotated, and it is shown that repeated restraining and rotating is more stressful for the animal. Therefore it can be argued that the negative experience is retained.

However, although distinct parameters such as white of the eyes or blood pressure, do show differences between treatments, there is no overall indication that one treatment (upright, 90 degrees or 180 degrees) is more stressful than the other. Further analysis on, for example heart rate variability, on these data is warranted.

Samenvatting

Momenteel is de kennis van het effect van het fixeren op het welzijn van runderen en kleine herkauwers beperkt; met name het effect van het positioneren en roteren van de dieren moet nader onderzocht worden. Bovendien is de informatie over de methoden en aantallen runderen, schapen en geiten die in de praktijk worden gefixeerd en geroteerd schaars. Ten gevolge van parlementaire discussies, heeft het Ministerie van Economische Zaken opdracht gegeven onderzoek te doen naar methoden voor het fixeren en roteren, met speciale nadruk op de positie van het dier (grote en kleine herkauwers) tijdens het fixeren en roteren. Het doel van deze studie was om inzicht te krijgen in de mate van stress die dieren ervaren tijdens verschillende methoden van fixatie en rotatie.
De impact van het rechtop versus geroteerde fixatie op het welzijn van dieren is beoordeeld op basis van gedrags- en fysiologische parameters. Het experiment is uitgevoerd op een testfaciliteit om standaardisatie van de procedure te vergemakkelijken. Het experiment is opgezet voor negen uitstoot melkkoeien. Alle negen dieren zijn blootgesteld aan drie verschillende behandelingen volgens een 3x3 Latijns vierkant. De behandelingen waren: 1) Fixatie rechtop, 0 graden rotatie 2) Fixatie gevolgd door 90 graden rotatie, 3) Fixatie gevolgd door 180 graden rotatie (omgekeerde positie / dorsale ligging). De rotatiefase duurde maximaal drie minuten. Tussen de experimentele dagen hadden de dieren een week om te herstellen. De hypothese was dat herhaalde blootstelling aan de fixatie box kan leiden tot ofwel gewenning of weerstand. Met een gerandomiseerde proefopzet kon het effect van de behandeling en eventuele gewenning gedetecteerd worden. Voor het meten van fysiologische parameters waren alle dieren voorzien van een chirurgisch geïmplanteerde telemetrische zender (Data Science International, DSI®, St Paul, MN), waarmee de lichaamstemperatuur, activiteit, bloeddruk en hartactiviteit (ECG) tijdens de experimentele periode geregistreerd konden worden. Voor gedragsparameters werden dieren voorzien van een niet-invasieve sensor voor het detecteren van herkauwen, eten en drinkgedrag (RumiWatch® (ITIN + HOCH; Ettenhausen, CH)) en activiteit werd vastgelegd met 3-dimensionale versnellingsmeters, meestal aangeduid als stappentellers, op 4 Hz (IceQube™ , IceRobotics Ltd, South Queensferry, UK). Daarnaast zijn ook gedragsobservaties uitgevoerd tijdens het opdrijven van de dieren naar fixatie box. Deze gedragsobservaties waren vocalisaties, weerstand om naar voren te bewegen, het tonen van oogwit, houding, ontlasting, urineren, draaien, en de manier van hanteren zoals stimulatie die nodig was om de dieren van de ene plaats naar de volgende plaats te laten gaan.
De resultaten tot nu toe laten zien dat er een significant effect is van eerdere ervaringen in de fixatie box op de benodigde tijd en de hoeveelheid stimulatie die nodig is om de dieren uit de startbox en naar de fixatie box te drijven ($F=5.5$, $p=0.022$ en $F=601$, $p<0.001$, respectievelijk). Dieren die niet eerder in de fixatie box waren geweest hadden minder tijd en aanmoediging nodig in vergelijking met dieren die al eerder in de fixatie waren geweest. Tijdens de fase in het experiment waarbij de dieren 0, 90 of 180 graden gedraaid werden was er een significant effect tussen behandelingen voor de gedragsparameter tonen van oogwit ($F=4.11$, $p=0.016$). Dieren die niet geroteerd werden (0 graden) toonden significant minder oogwit ten opzichte van dieren die 90 of 180 geroteerd werden. Het verloop van hartslag in de tijd verschilde significant tussen de behandelingen ($F=2.85$, $p<0.001$); terwijl de hartslag van de dieren die niet geroteerd werden tussen de 88 en 95 slagen per minuut lag, daalde de hartslag van de dieren die geroteerd werden tot ongeveer 76 tot 80 slagen per minuut. Hoewel er geen effect van de behandeling was op het verloop van de diastolische bloeddruk tijdens de drie minuten behandeling (rotatie 0, 90, 180 graden) was er wel een significant effect van eerdere ervaringen in de fixatie box. Bij dieren die eerder in de fixatie box waren geweest (in dit experiment) werd een significante daling van de diastolische bloeddruk ($F=2.66$, $p=0.047$) tijdens de periode van drie minuten in de fixatie en rotatie box waargenomen, ongeacht de behandeling. Om het effect van de behandeling op het herstel te bestuderen, werden lig- en herkauw activiteit vastgelegd. Er was geen effect van de behandeling op zowel de latentietijd om te voor het eerst te gaan liggen of herkauwen als op de duur van de eerste periode liggen of herkauwen.

Samenvattend, laten de resultaten van deze studie zien dat fixeren en roteren belastend is voor de koeien. Uit de resultaten valt op te maken dat roteren acute stress oplevert, en dat wanneer een fixatie en rotatie herhaald wordt dat nog meer belastend is voor de dieren. Daarom kan worden gesteld dat de negatieve ervaring wordt onthouden .
Hoewel afzonderlijk parameters, zoals oogwit of bloeddruk, verschillen laten zien tussen de behandelingen, is er geen generieke indicatie dat een specifieke behandeling (rechtop, 90 graden of 180 graden) meer belastend is dan de andere. Verdere analyse van bijvoorbeeld hartslagvariabiliteit op deze gegevens is daarvoor nodig.

1 Introduction

Arising from the debate concerning the welfare of animals before and at slaughter, the Dutch Ministry of Economic Affairs ratified a motion on "Improving the welfare of slaughter animals". Furthermore, in the covenant on slaughter without stunning, the Dutch Ministry of Economic Affairs has, in close compliance with concerned parties, listed areas requiring further research to help safeguard and improve animal welfare during slaughter without stunning without stunning (Agreement on unstunned slaughtering in accordance with religious rites, June 2012). These areas include:

1. Identification of critical control points
2. Validation of parameters to examine unconsciousness
3. Reduction in time between neck cutting and loss of consciousness
4. Number of neck cuts required
5. Method and duration of restraint

As a response to parliamentary discussions, the Ministry of Economic Affairs has commissioned a study into methods of restraint, with special emphasis being accorded to position of the animal (large and small ruminants) during restraint.

At present, knowledge concerning the effect of current restraining methods on the welfare of cattle and small ruminants in is limited; and especially the impact of positioning and rotating the animal warrants further study. In addition, information is scarce concerning the methods and numbers of cattle, sheep and goats that are restrained and rotated in practice.

At slaughter animals have to be transferred from lairage to the slaughter area. Animals generally enter the slaughter area through a race. In order to facilitate slaughter without stunning and to protect operational personnel some kind of additional restraint is required (Peeters, 2007; Anil, 2012). This may take various forms, e.g. a rotation box, a conveyor belt-type system, manual restraint or handling of standing animals in an open area or pen. Safety and welfare of both animals and slaughterhouse personnel are primary concerns especially when restraining larger animals.

Animal welfare can be defined as the quality of life as perceived by the animals themselves (Bracke et al., 1999a). In relation to restraint during un-stunned slaughter, for example, it is very important to realise that what may look awful to a human observer (e.g. blood, convulsions) doesn't have to be an indication of negative welfare of the animal. Convulsions may occur after the animal has lost consciousness.

Related to the main welfare needs (pain, safety, social contact and movement) the following welfare aspects and risks may have to be considered when evaluating the welfare implications of restraining methods for slaughter without stunning:

- Pain: Pain is related to injury, which may arise when animals are moved into and out of the restrainer (e.g. use of electric goads, slipping, colliding with the restraining device, hoisting). Pain experienced while in the restrainer may also arise from various sources, e.g. from handling, poorly fitting equipment, neck cutting, wound touching and struggling. During slaughter pain reactions may be masked by the restraining device or when the animal is shackled (Holleben, 2007).While neck cutting may be considered to be a separate welfare issue it relates to restraint in that the method of restraint may affect the ability to execute neck cutting or humanitarian interventions such as emergency stunning.

- Safety: The need for safety relates to the perception of fear. It involves both fear of external stimuli and fear related to social interaction, i.e. aggression. The perception of fear is related to unpredictability, e.g. due to unfamiliarity, and an important behavioural strategy to reduce fear is habituation. Fear-evoking stimulation may be visual (darkness/bright spots; animal movements),

auditory (distress calls), tactile (sudden touches) and olfactory (e.g. the smells of blood, viscera, burning or stress of other animals, cf Jones and Nowell, 1973; McGlone, 1990). A particularly fear-evoking condition is likely to be induced by rotation, due to the unfamiliarity with this position and its evolutionary significance.

- Social contact: This need relates to stress responses resulting from social isolation while in the restrainer as well as to social factors that may mitigate fear responses, e.g. the use of so-called 'leader sheep' (an individual sheep that has been familiarised to the conditions in the slaughterhouse and is used to lead other sheep into the slaughter area).

- Movement: The ability to move is an important aspect of welfare. It is especially limited during restraint. The need to move relates, for example, to space requirements for farmed animals during normal rearing conditions. Animals that are accustomed to be able to move freely (e.g. cattle kept in yard systems) may experience more stress when confined in a restraining box compared to cattle which are accustomed to being handled and tethered.

To assess the welfare implications of methods of slaughter without stunning both behavioural and physiological parameters can be used.

For behaviour parameters such as resistance to move, posture, vocalisations (Manteuffel et al., 2004), urinating, defecating have been used in numerous studies assessing animal welfare . Additionally, studies have indicated that wide-open eyes might be an emotional indicator in cows (Sandem et al., 2002). In a further study Sandem et al. (2006) demonstrated that an increased percentage of eye white reflects frustration in dairy cows.

For the physiology the autonomic nervous system plays in important role. In farm animals, the vagal component of the autonomic nervous system plays a key role in regulating heart rate in response to stress (Hopster and Blokhuis,1994; Hansen and von Borell, 1998). In the past decades several hear rate studies focused on the effect of stress of the animals related to separation (Boissy and Le Neindre, 1997), weaning (Hopster et al., 1995), veterinary procedures (Waiblinger et al., 2004), early handling (Stewart et al., 2013), social interactions (Laister et al., 2011) and human contact (Rushen et al., 1999; Schmied et al., 2008a and 2008b). Moreover, more recent studies analysing heart rate variability for assessing the level of stress have become prominent in livestock species (Kovacs et al., 2014). Measuring blood pressure may also be an indicator to assess physiological stress in ruminants. For example in experiments in sheep showed a rise in blood pressure and increased heart rate with an increase in rumen pressure (c.f. Dougherty, 1940)

In some countries the slaughter of cattle without stunning in dorsal recumbency is prohibited (Grandin, 1995) because of the stress involved in inverting the animal (Dunn 1990, Grandin & Regenstein 1994, Shragge & Price 2004). In the EU, Member States Slovakia, Denmark and United Kingdom prohibit the use of rotating or inverted restraint pens for use for slaughter without stunning.

Rotary designs are widely used in many other EU Member States and are currently allowed under EU Regulation 1099/2009. However, article 27 of the regulation states that the council had commissioned a report, (submission in December 2012) on systems of restraint for bovines by inversion or any unnatural position. An EU commissioned report is envisaged, based on the results from a scientific study comparing rotation and upright systems of restraint. The intended report shall probably contain legislative proposals for (further) amendments to regulation no. 1099/2009.

The above mentioned scientific study has been performed under the acronym BoRest. The author of the present report has, on behalf of Wageningen UR Livestock Research, participated in this study. The report of the BoRest study has discussed by a panel of experts and should be available later this year..

Objectives

The aims of the current national project "Research into welfare of ruminants during restraining at slaughter without stunning" (BO-20-008-005.01) is:

1. To obtain information concerning the current practice of restraining animals at slaughter (phase 1).
2. To gain insight into the levels of stress animals encounter during different methods of restraining (phase 2 and 3).

Results of the first objective (phase 1) have been described in the progress report "Review into restraint of ruminants during un-stunned slaughter" delivered in April 2013.

An experiment with cattle (culled cows) was devised to improve our insight into the levels of stress animals encounter during restraining (phase 2). This progress report describes the experiment and discusses the results from which several conclusions arise.

2 Materials and methods

Impact on animal welfare of up-right versus inverted restraining was assessed based on behavioural parameters and physiological parameters. The experiments have been carried out under controlled circumstances at the research facilities of Wageningen UR Livestock Research with the approval of the ASG ethical committee on animal experiments. Conducting this experiment on our test facility was preferred instead of conducting it at a commercial slaughter location in order to avoid environmental bias, and transportation and to facilitate standardisation of the procedure.
Results of this study will be used to design a second experiment under (semi-) practical conditions.

2.1 Experimental design

An animal experiment had been designed in order to gain insight into the effect of restraining and rotation to complete inversion. Nine culled dairy cows were used as experimental animals. These animals have been exposed to 3 different treatments following a 3x3 latin square design.

Treatments:
- Restraining in upright position
- Restraining followed by 90 degrees rotation
- Restraining followed by 180 degrees rotation (inverted position/dorsal recumbence)

An efficient randomized design ensured that a minimum number of animals (9) were exposed to all 3 treatments during the experiment. The experimental design was devised assuming a between-treatment detection level of 1.5 times the SD, at a significance level of 0.05, with a power of 0.80, using a two-sided test (see 2.6 Statistics).

Limitations of available equipment (transmitters) and the large impact of the surgical and experimental procedures on the animals justified the limited number of animals in the experiment. Therefore, if the outcome and variation in the data provide sufficient evidence a second experiment could be avoided.

The animals were allowed a week to recover between treatments. It is hypothesized that repeated exposure to the restraining pen may lead to either habituation or aversion. The randomized experimental design helped to detect the treatment impact on the animals and any habituation to the treatment.

Repeated use of the animals has the advantage to compare treatments within cows. Different cows may have different levels of body parameters, such as heart rate of blood pressure. A latin square design gives the opportunity to eliminate these constant cow effects and have a more powerful comparison of treatment effects. Also disturbing time effects (days of measuring) can be eliminated in the analysis.

The use of 9 different cows with different backgrounds (history, age) gives a fair picture in terms of exploration of the results to a larger scale. However, this was not investigated thoroughly.

The actual experiment in which the treatments took place was divided in phase 0 – phase 7:
- Phase 0: Basal recordings were taken in the starting pen (waiting area) for approximately 3 minutes
- Phase 1: Animals were driven out of the starting pen
- Phase 2: Animals were driven onto the bridge towards the entrance of the restraining pen
- Phase 3: Once in the restraining pen, a second basal recording (1 minute) was made while the animals were standing still without being restrained
- Phase 4: Animals were being restrained

- Phase 5: Under treatment (180 seconds): animals were either not rotated or rotated to 90 or180 degrees.
- Phase 6: Animals were released
- Phase 7: Animals were driven out of the restraining pen.

2.2 Animals

Nine culled dairy cows (Holstein Friesian, average age 65.1 ± 20.8 months) were selected from the herd at Wageningen UR Dairy Campus (location Lelystad). All animals were in normal condition without clinical indication of health problems. One to three days prior to surgical placement of the transmitters, the animals were taken from the herd and housed together in a tie-stall, where they remained for the duration of the experiment (i.e. 6 weeks). Animals were fed a standard diet for non-lactating cows and had *ad libitum* access to drinking water. No physical contact was possible between cows (1 meter between each tied stand) but were able to hear, see and smell each other. After completion of the experiment the cows were transported to a slaughter house where they were slaughtered.

2.3 Sampling systems

2.3.1 DSI Telemetry

Approximately 1 to 2 weeks prior to the first restraining day, all cows were surgically implanted with a telemetry transmitter (Data Science International, DSI®, St Paul, MN) allowing registration of body temperature, activity, blood pressure and cardiac activity (ECG) during the experimental period. During surgery, each animal was placed in a claw trimming box, with a hammock under the abdomen to prevent them from laying down. The cows were sedated with 0,8 ml Domidine® and the surgical site was locally infiltrated with 100 ml Lidocain 2% solution. The body of the transmitters was placed subcutaneously (SC) in the left jugular groove. The systemic blood pressure catheter was inserted through a purse string suture in the carotid artery towards the cranium (approximately 15 cm). The positive ECG lead was fixated close to the transmitter. The negative ECG lead was inserted into the jugular vein through a purse string suture in a caudal direction with its solid tip as close to the heart as possible (but not touching the heart). Purse string sutures were performed using prolene™ 3-0, with an a traumatic cardiovascular needle. The subcutis and skin were closed with vicryl-0® (Ethicon). Post-surgery, each cow was treated with Depocilline® (1ml/25 kg bodyweight, MSD Animal Health) and Finadyne® (2 mg/kg bodyweight, MSD Animal Health) . Rectal temperature was measured daily. No wound infections occurred and rectal temperature remained between 38-39°C in all cows during the experimental period. One cow received an extra three-day treatment of Finadyne® for lameness, this was not related to surgery. All animals had recovered from surgery within a week and surgery wounds healed within 10 days.

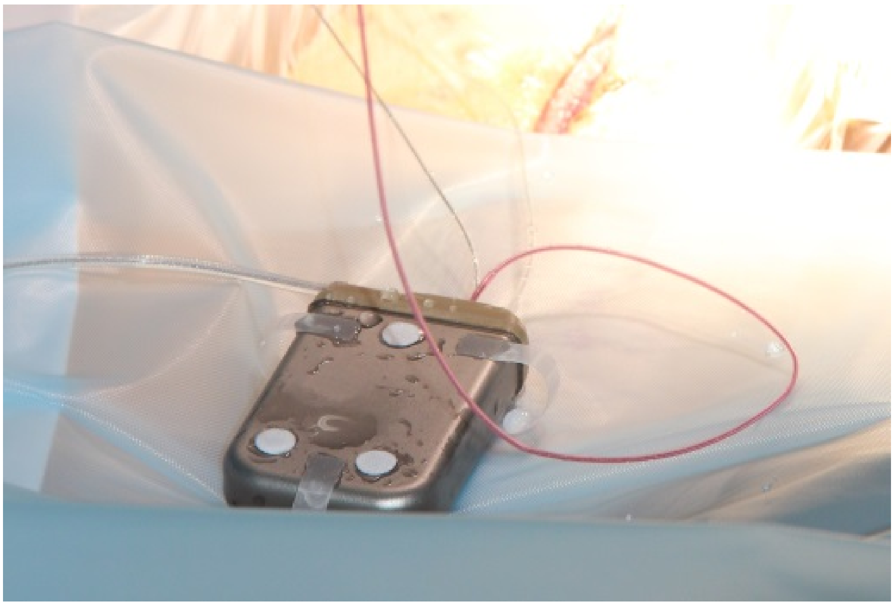


Figure 2.1 *L11 transmitter model (DSI®)*

All data were recorded continuously with a frequency of 500 Hz using the telemetry system of Data Science International, DSI®, St Paul, MN; it consisted of:

- Implantable transmitters (PhysioTell™ Digital, Model L11) with two biopotential leads (positive lead and negative solid tip lead) for measuring ECG signal, a temperature sensor to measure body temperature and a blood pressure fluid filled catheter (technical features: weight 56 grams, dimensions: 59*38*15mm, battery life 15 weeks, transmission range 3-5 m)
- 12 Transceivers (TRX-1, DSI® for Large Animals).
- Ambient pressure reference (APR-1)
- Ethernet to Serial Converter for APR-1 (E2S-1)
- 2 CLC's (Communication Link Controller)
- Cisco Gigabit Security Router
- PC with Ponemah Software System version 5.2 (with an acquisition-, an analysis- and operating-room-system)

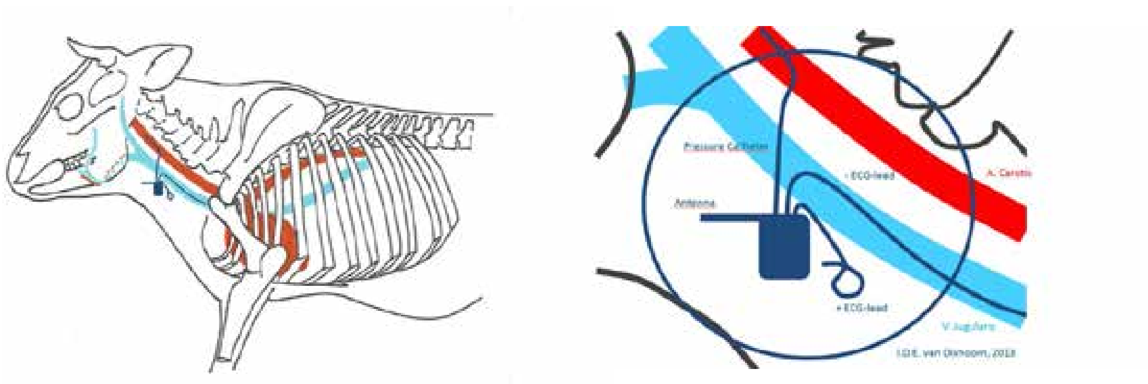


Figure 2.2 *Schematic picture of transmitter placement (left) and zoom (right)*

2.3.2 Rumiwatch®

All animals were equipped with a non-invasive sensor to determine ruminating, eating and drinking behaviour. The RumiWatch® (ITIN+HOCH; Ettenhausen, CH) system consists of a noseband sensor (NBS). The NBS comprises a vegetable oil-filled tube, pressure sensor and accelerometer, and a wireless transmitter. Jaw movements are registered at a frequency of 10 signals per second. Data analysis is conducted in real time via validated algorithms of RumiWatch® electronics.



Figure 2.3 *RumiWatch system, copyright ITIN+HOCH*

2.3.3 IceQube™

Activity was recorded with 3-dimensional accelerometers, commonly referred to as pedometers, at 4 Hz (IceQube™, IceRobotics Ltd., South Queensferry, UK). Throughout the study period, each cow was equipped with an IceQube which was attached to the fetlock of the left hind leg. IceQubes continuously determined whether a cow was laying or standing, and recorded the number of steps per 15 minute period. After the study, data from IceQubes were transferred by IceReader interface to a computer equipped with IceManager software. These data were then used to derive the laying and standing bouts of the test cows.



Figure 2.4 *IceQube on hind leg*

2.4 Restraining pen

For this experiment, a commercial available rotating restraining pen (MPS Red Meat Slaughtering, Lichtenvoorde, The Netherlands) was used. In this pen animals of different sizes can be fully restrained using the adjustable back pusher, sidewall and top plate, breastplate and chin lift.



Figure 2.5 *Restraining and rotating pen*

This restraining pen was capable of restraining animals in any position between standing (0 degrees) and fully inverted (180 degrees). Level of restraining, i.e. the pressure of the movable side plates as well as the turning position were set manually using a control panel (Figure 2.6) that activated an hydraulic pump.



Figure 2.6 *Rotating a cow*

Entrance of the restraining pen was 0.8 meters above ground level. A 4 meter long wooden corridor was constructed with a slope of 11.5 degrees to overcome the difference between floor level and the entrance of the restraining pen. The floor surface of the wooden race was covered with a non-slip rubber mat. The rotation speed was 11.5 seconds for 360 degrees rotation. So the animals were rotated 90 degrees at 2.9 seconds and 180 degrees at 5.8 seconds.

2.5 Measurements

The effect of standing restraint (rotation 0 degrees) versus rotation 90 or 180 degrees was judged based on behavioural and physiological parameters measured during the restraining experiments. Recovery and or habituation or aversion were judged based on behaviour and physiological response observed in the period between restraint days and during driving and entrance into the system on the subsequent experiment days.

2.5.1 Behaviour

Video recordings were made on the restraining days to monitor behaviour prior to, during and after the restraining period. From these video recordings resistance behaviour in the starting pen, entering the restraining pen, and in during the rotation phase were assessed. The following behavioural parameters were included in the ethogram:

Table 2.1
Parameters used to assess behavioural responses during phases 0-7

Parameter	Classification
Duration	Time (in seconds) for a particular phase
Stimulation	Stimulation given by the handlers: 1=no stimulation needed; 2=moderate stimulation by voice needed; 3=abundant stimulation, repeated physical contact needed
Use of aids	1=voice, 2=physical contact (with hand), 3=use of a prod
Turning	1=no turning/no attempts, 2=attempt to turn/escape, 3=turn/escape
Posture	1=standing, 2=laying down/sitting
Vocalisations	1=no vocalisation, 2=lowing, 3=groaning
Resistance	1=standing still, 2=moving legs, trying to regain posture, 3=kicking
Defecating	Number
Urinating	Number
White of the eyes	1=normal, almost no white visual; 2=little, eye white visible at one corner of the eye; 3=much, eye white visible around a large part of the cornea; 4=much and protruding eyes
Leaving the restrainer	1=without help, 2=guidance is needed, 3=not backwards but to the side

Furthermore, recovery to normal activity was assessed on activity, i.e. laying periods and ruminating activity. Firstly, the latency to first laying/rumination bout was recorded. Secondly, the total time of the first laying/rumination bout was used as an indicator.
A rumination bout was defined as a period in which the animal ruminated for at least 10 minutes without interruption after being returned to the stable . For laying down, no such definition applied; each laying activity was scored as a laying bout.

2.5.2 Heart rate, body temperature and blood pressure

Heart rate, body temperature and blood pressure were recorded continuously during phase 0-7 of the experiment. Apart from that, base-line levels were recorded during a selected period between the restraining days.
The continuous measurements of ECG (heart rate), blood pressure, activity and body temperature were used to determine the physiologic changes during fixation and rotation as compared to the physiological parameters during the period prior to the rotation and fixation (a period of 3 minutes waiting before entering and the period of the movements in the corridor towards the restraining pen)

Both heart rate and blood pressure data was analysed for all phases of the experiment, but in more detail for the 180 seconds of the treatment (phase 5): heart rate and blood pressure were analysed for 6 consecutive periods of 30 seconds.
Furthermore, the results of the blood pressure were analysed for a) diastolic blood pressure and b) the difference between systolic and diastolic blood pressure (Δ).

2.6 Statistical analysis

Behaviour and handling data as well as data of rumination activity and laying behaviour individual measurements of each cow-week combination (27 different measurements, see table 2.2) were analysed simultaneously in a latin square analysis, were treatment effects were corrected for random cow and week effects. As habituation or aversion for repeated exposure may occur, the analysis was expanded with model terms to correct treatment effects for residual (or carry over) effects of the previous treatment.

As behaviour data were mainly scores, these data were considered as ordinal data. Other data were normalized by the using the log-transformation. Blood pressure and heart rate data were analysed with a longitudinal model to detect different slopes, where correlation of measurements of the same cow within a day was estimated. Influence of carry over effects of the previous treatments were also estimated within this latin square longitudinal analysis.

Table 2.2
Cow nr and treatment order

Cow nr	1st time	2nd time	3rd time
5778	0 degrees	90 degrees	180 degrees
4903	180 degrees	0 degrees	90 degrees
5611	90 degrees	180 degrees	0 degrees
5754	90 degrees	0 degrees	180 degrees
5187	0 degrees	180 degrees	90 degrees
3672	180 degrees	90 degrees	0 degrees
5864	180 degrees	0 degrees	90 degrees
5197	0 degrees	90 degrees	180 degrees
6066	90 degrees	180 degrees	0 degrees

Assessment of treatment effects on animal behaviour and physiology did not solely rely on distinct differences between treatments but also whether or not animals had previous experience of rotating within the current experiment.

3 Results

The results of the present study have been compiled in four sections:
- Behaviour and handling before and during treatment
- Heart rate before and during treatment
- Blood pressure before and during treatment
- Behaviour (laying behaviour and rumination) after the treatment in the "home" environment

3.1 Behaviour and handling before and during restraining

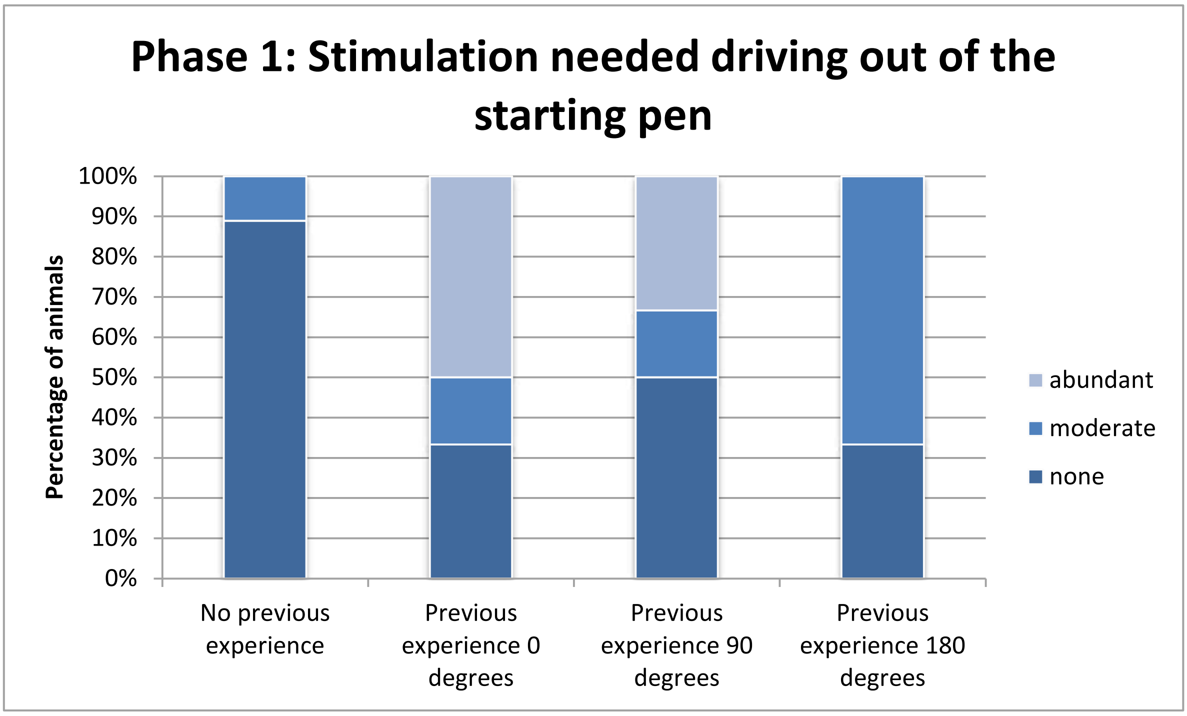


Figure 3.1.1 *The amount of stimulation required to drive the animals out of the starting pen, with different previous experiences*

In figures 3.1.1 and 3.1.2 the amount of stimulation required to drive the animals from one area to the next is presented as the percentage of animals with or without previous experience in the restraining pen.
The amount of encouragement/stimulation required to drive animals out of the starting pen with no prior experience of the restraining pen was significantly lower compared to driving those with prior experience (figure 3.1.1; $F=601.4$; $p<0.001$). No such significant difference was found for driving the animals into the restraining pen(figure 3.1.2).

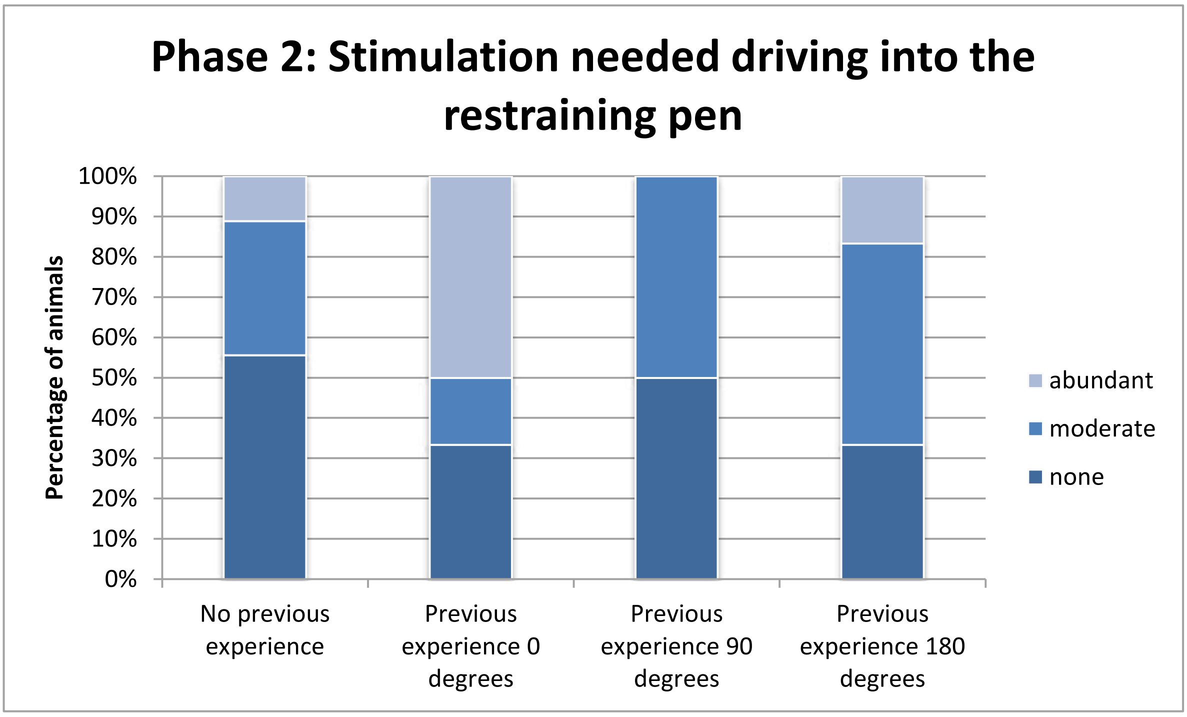


Figure 3.1.2 *The amount of stimulation required to drive the animals from the starting pen towards the restraining pen, with different previous experiences*

The type of stimulation used by the researchers (voice, touch, stick, prods) did not differ significantly with prior experience of the individual animal.

In figure 3.1.3 presents an indication of the time required (in seconds) as recorded for the phases 1 and 2 (respectively driving the animals out of the starting pen and into the restraining pen). Statistical tests indicated a significant effect of previous experience on time required to drive the animals out of the starting pen (phase 1: $F=5.5$, $p=0.022$). Animals without previous experience together with those with previous experience of 180 degrees took significantly less time compared to animals with previous experience of 0 degrees and 90 degrees of rotation. For the second phase, no such significant difference was apparent.

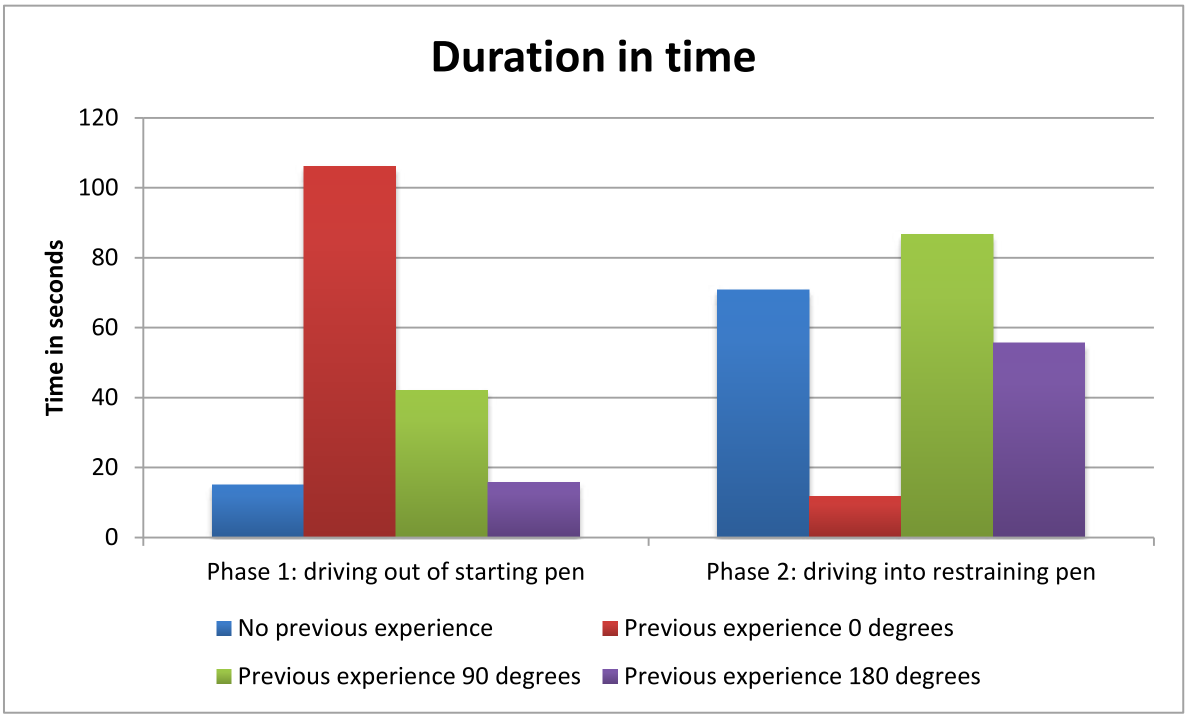


Figure 3.1.3 *The duration (in seconds) required to drive the animals from one area to another (Phase 1 and Phase 2)*

In phase 5 of the experiment, it was planned that each individual would receive its previous randomly decided treatment: 0 degrees rotation, 90 degrees rotation or 180 degrees rotation. Phase 5 was executed 3 times for each individual, resulting in (3 x 9 =) 27 treatment times. From these 27 treatment times, phase 5 was incomplete due to certain irregularities (laying down, too stressed) or problems with the rotation mechanism. Therefore, data is missing for several treatment times in phase 5 . For day 1 all data was available. On day 2 during the treatment 90 degrees rotation of cow 5197 the rotating system failed and data in phase 5 is therefore missing. For the treatments '0 degrees' (cow 3672) and '90 degrees' (cow 5864) on day 3 phase 5 never started because the cows immediately were laying down after entering the rotation pen. Both animals refused to remain a standing position in the rotation pen and are therefore abandoned from the analyses. Additionally, in 3 cases all on day 2, phase 5 was aborted within 180 seconds, for the '90 degrees' treatment cow 5778 was returned to standing position after 1 minute due to miss communication between operators. In treatment '0 degrees' cows 5754 and 5864 collapsed or tried to lay down during the fixation period. Cow 8754 was hyperventilating during this collapse. Both tests were stopped immediately at this point at respectively 132 and 120 seconds. However, in these instances, the available data for this phase was used in the analysis.

The animals displayed some resistance: moving with the legs and kicking during the 3 minutes of treatment (figure 3.1.4). However, no significant treatment effect was indicated on this behavioural parameter. Similarly, no significant effect of previous experience was observed (data not shown).

Additionally, some animals prominently displayed the whites of their eyes. The percentage of animals showing more whiter of the eye differed significantly between treatments (F=4.11, p=0.016). Animals restrained without rotation (rotation 0 degrees) displayed significantly less white of the eye compared to the other two treatments (figure 3.1.5). Statistical analysis did not reveal a significant effect of previous experience for this parameter (data not shown).

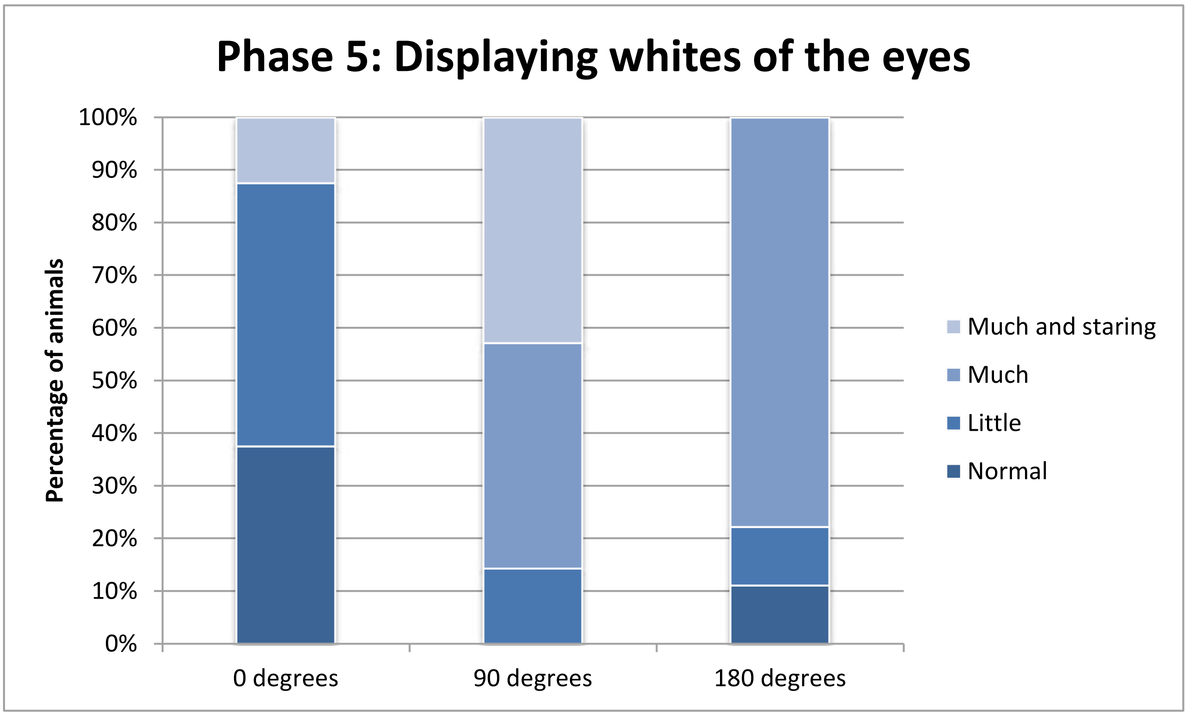


Figure 3.1.4 *Percentage of animals displaying resistance during treatment phase 5*

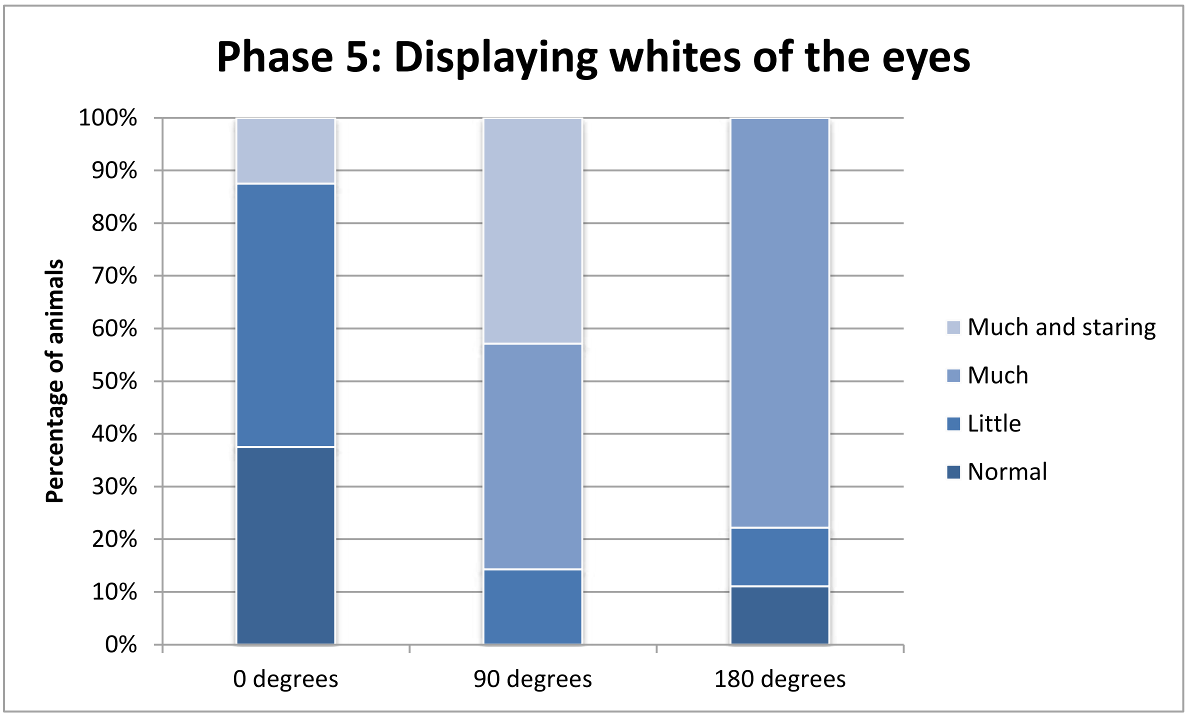


Figure 3.1.5 *Percentage of animals displaying the whites of their eyes during treatment phase 5*

Although animals displayed attempts to escape and to turn around during all treatments, statistical analysis did not indicate significant differences.

Urinating and defecating occurred very little (almost none). Vocalisations occurred predominantly in the rotational phase with an even distribution across treatments of lowing and groaning. Similarly, the distribution of evasive behaviour indicators (leg movement and kicking) was evenly distributed over treatments (see appendix 1). No effects of previous experiences were discovered.

3.2 Heart rate before and during treatment

See for an illustration of heart rate and blood pressure of an individual cow, being rotated 180 degrees appendix 2.

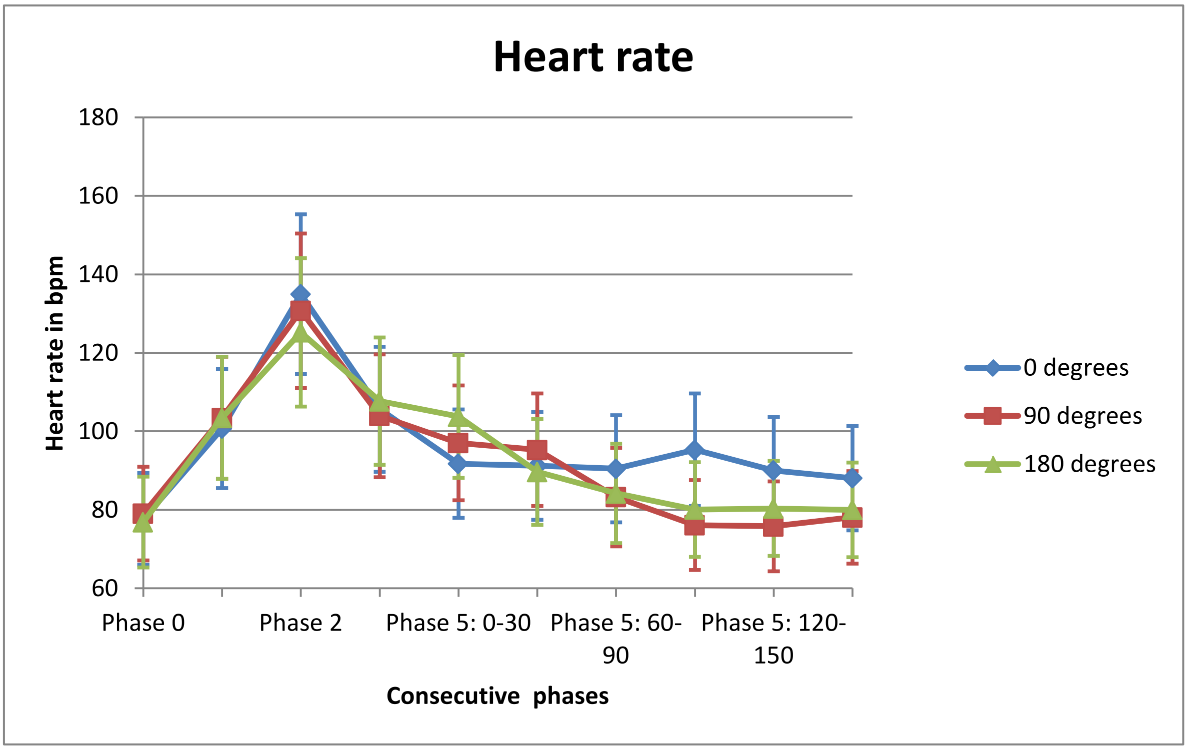


Figure 3.2.1 *Mean heart rate (in bpm) per treatment during the consecutive phases 1 through 5; phase 5 being subdivided into 6 consecutive periods of each 30 seconds*

Figure 3.2.1 shows the pattern of mean heart rate for all 9 animals per treatment. While the mean heart rate starting pen (phase 0) resides around 78 beats per minute, this increases to maximum values between 125 and 135 bpm during driving into the race and towards the restraining pen (phase 2). Once inside the restraining pen, the mean heart rate for all treatments is around 106 beats per minute. During treatment (rotation: 0, 90, 180 degrees) heart rate patterns over time differ significantly between treatments ($F=2.85$, $p<0.001$); whereas the heart rate of animals restrained without rotation resides between 88 and 95 beats per minutes, for rotated animals the heart rate decreases to about 76 to 80 beats per minute after a minute in a rotated position.

Figure 3.2.2 illustrates the comparison of the mean heart rate before and during treatment with consideration of previous experience of the restraining pen. No significant differences were found in mean heart rate before and during treatment for animals with or without previous experience.

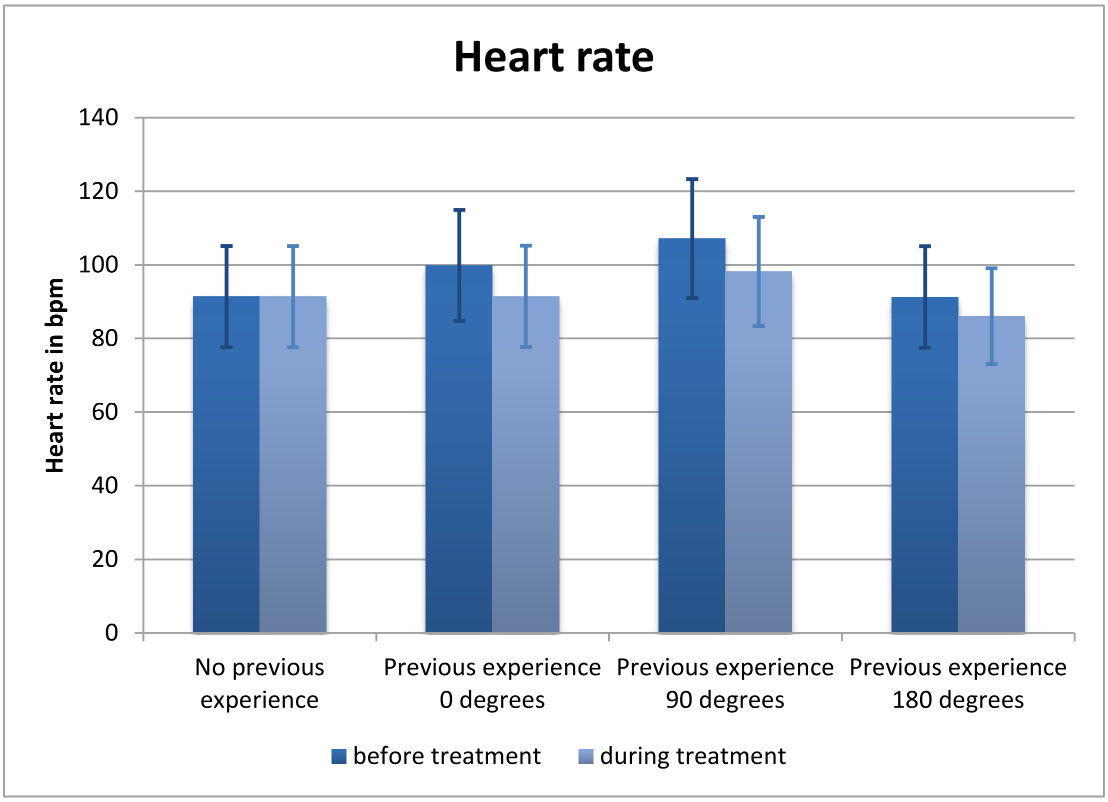


Figure 3.2.2 *Mean heart rate before and during treatment for animals considering previous experience of the restraining pen*

3.3 Blood pressure before and during treatment

3.3.1 Results for diastolic blood pressure

Firstly, figure 3.3.1 shows the mean pattern of diastolic blood pressure for 9 individuals for the different treatments. During phase 0, in the starting pen the mean diastolic blood pressure was between 128 and 135 mmHg. Diastolic blood pressure increases during driving until phase 3 during which the animals are standing still in the restraining pen. Thereafter, the patterns of the diastolic blood pressure differ slightly over time. While the diastolic blood pressure increased when animals were rotated 180 degrees, it fell during the first 30 seconds of the 0 and 90 degrees treatments. Thereafter, it increases to levels similar to those observed during the 180 degrees rotation treatment. These patterns do not differ significantly between treatments.

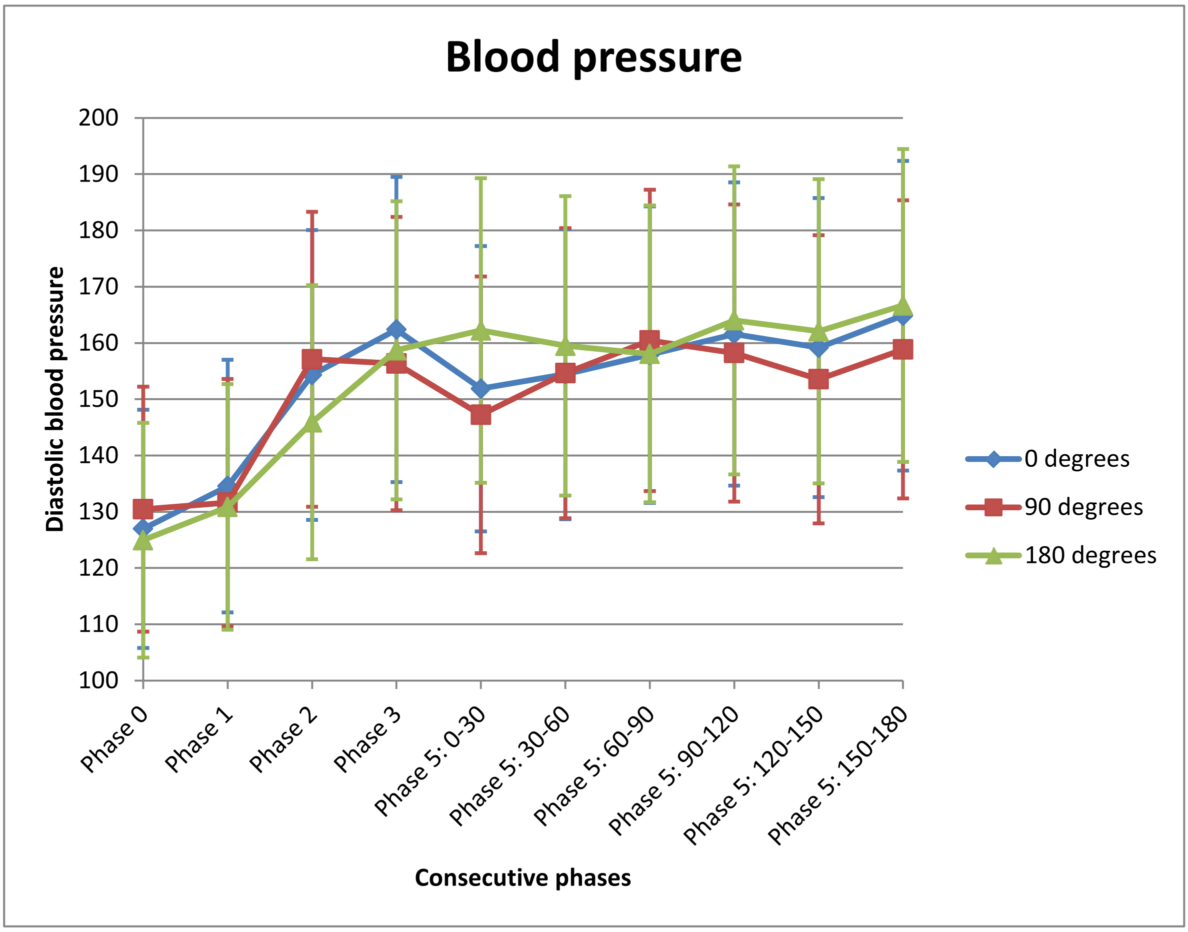


Figure 3.3.1 Mean diastolic blood pressure per treatment for each phase of the experiment, phase 5 being subdivided into 6 consecutive periods of each 30 seconds

The above mentioned effect can also be illustrated as average blood pressure before and during the treatment (figure 3.3.2). Again, it is shown that the diastolic blood pressure before treatment (rotation) is lower compared to the diastolic blood pressure during treatment; and that small differences between treatments exist. However, the effect of the treatment (rotation) was not significant for the type of treatment (rotation 0, 90, 180 degrees).

Secondly, the effect of a previous experience in the restraining pen was analysed. In figure 3.3.3 it is shown that the animals without previous experience of being in the restraining pen had a diastolic blood pressure of around 160 mmHg, while animals with experience had a slightly higher diastolic blood pressure. During the treatment, animals without earlier experience in the pen displayed the same diastolic blood pressure, but animals with experience showed a significant drop in diastolic blood pressure ($F= 2.66$ $p=0.047$).

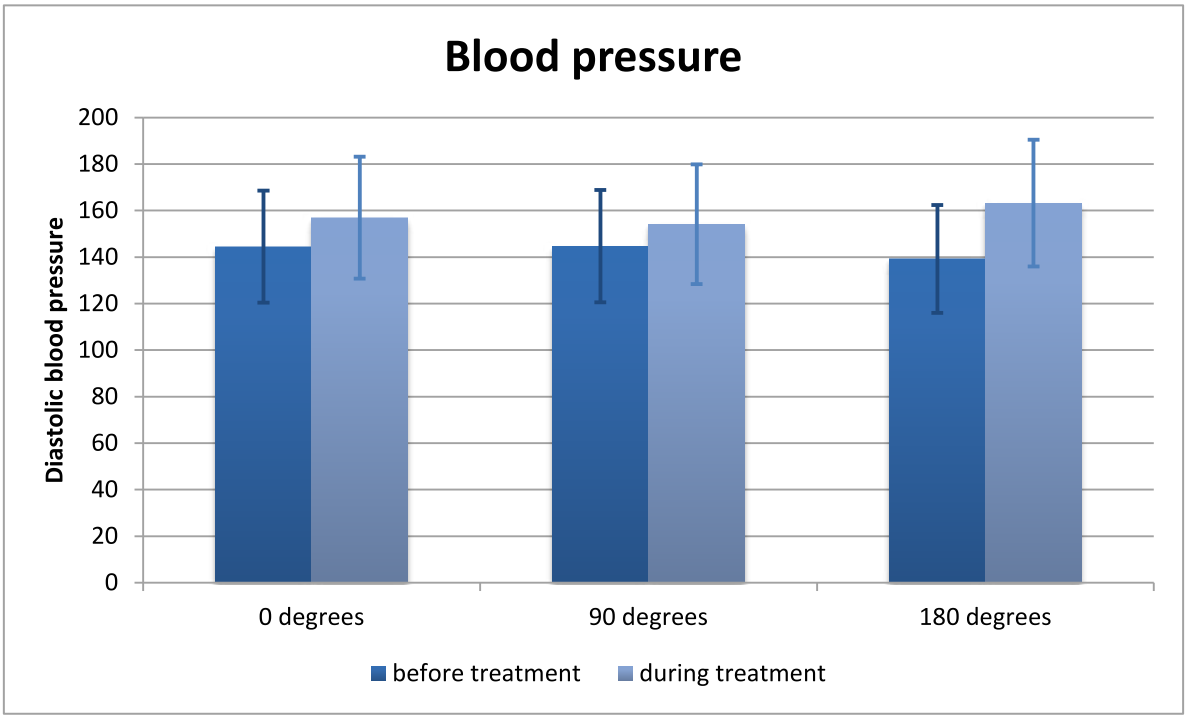


***Figure 3.3.2** Mean diastolic blood pressure before and during treatment*

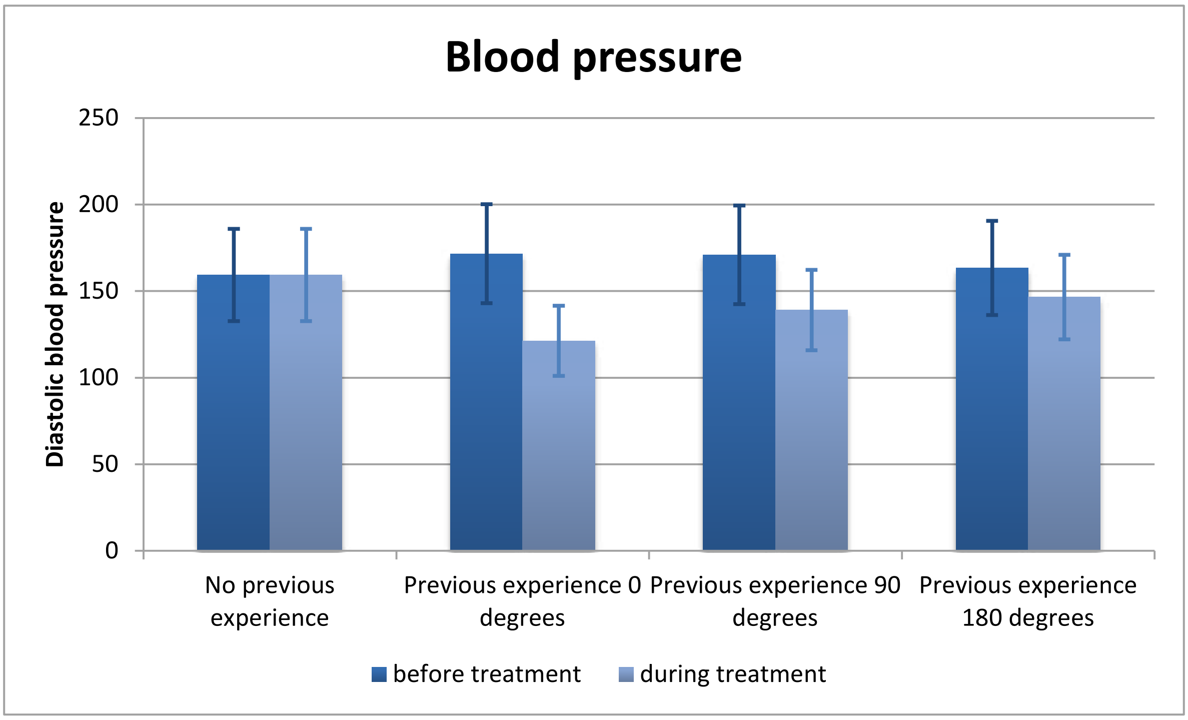


Figure 3.3.3 *Mean diastolic blood pressure before and during treatment with and without a previous experience in the restraining pen*

3.3.2 Results for the Δ blood pressure (difference between systolic and diastolic blood pressure)

Difference between systolic and diastolic blood pressure, Δ blood pressure, was calculated to measure the relative increase or decrease in blood pressure aspects.
For blood pressure, the following aspects were analysed in more detail: pattern of the three treatment groups over time; treatment effects on the Δ blood pressure before and after treatment application, and whether or not previous experiences affected the Δ blood pressure before and after the treatment.

Firstly, the pattern in the average Δ blood pressure (calculated as systolic blood pressure minus diastolic blood pressure) is shown in figure 3.3.4. The Δ is around 60 mmHg during the basal measurement in the starting pen (phase 0) and the Δ increases to reach maximum values of about 80 mmHg during phase 3 (standing still in the restraining pen). Thereafter, there the Δ blood pressure becomes much smaller for the 90 degrees rotation treatment, resulting in 35 mmHg. A much smaller decrease is found for the 0 degrees and 180 degrees rotation treatments, remaining large, approximately 80 mmHg. Regarding phase 5 (0-180 seconds treatment) the pattern over time displays a trend towards significant differences between treatments (F=1.72, p=0.078).

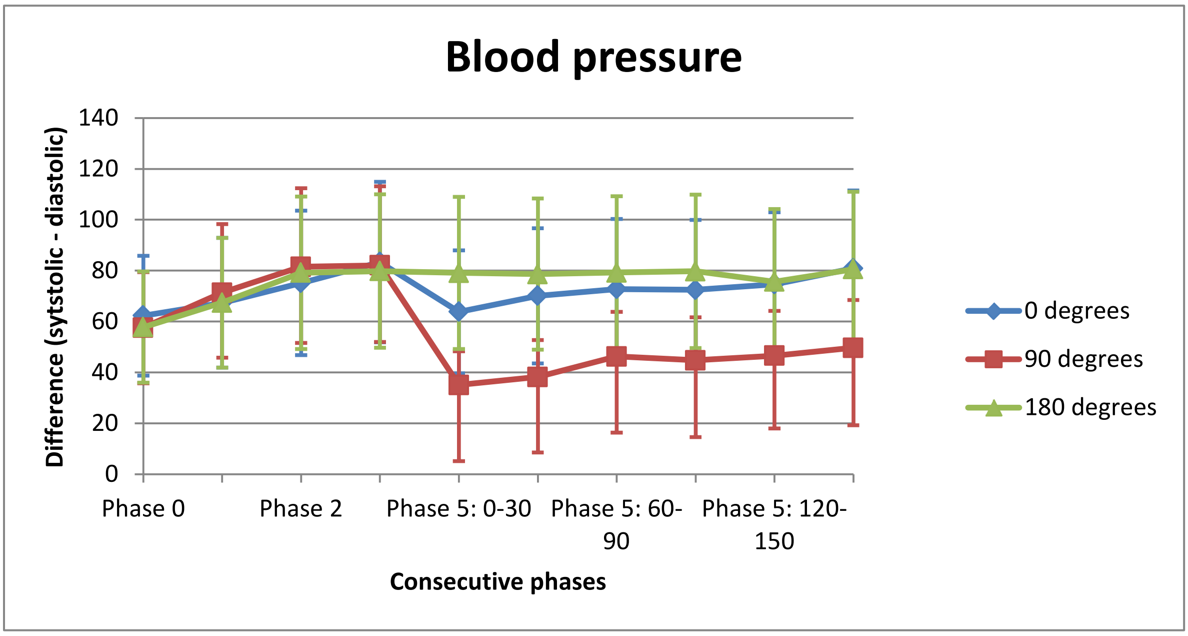


Figure 3.3.4 *Δ blood pressure (systolic minus diastolic) during the consecutive phases of the treatments, phase 5 being subdivided into 6 consecutive periods of each 30 seconds*

This effect of treatment can also be illustrated with figure 3.3.5. Comparing the levels before and during treatment it was concluded that there was a significant effect on the Δ(systolic minus diastolic) blood pressure (F=5.67, p=0.016). The Δ blood pressure (systolic-diastolic) was around 70 mmHg before treatment. During treatment, this Δ was maintained around these levels for the 0 degrees treatment, it increased slightly for the 180 degrees rotation and fell significantly for the 90 degrees rotation.

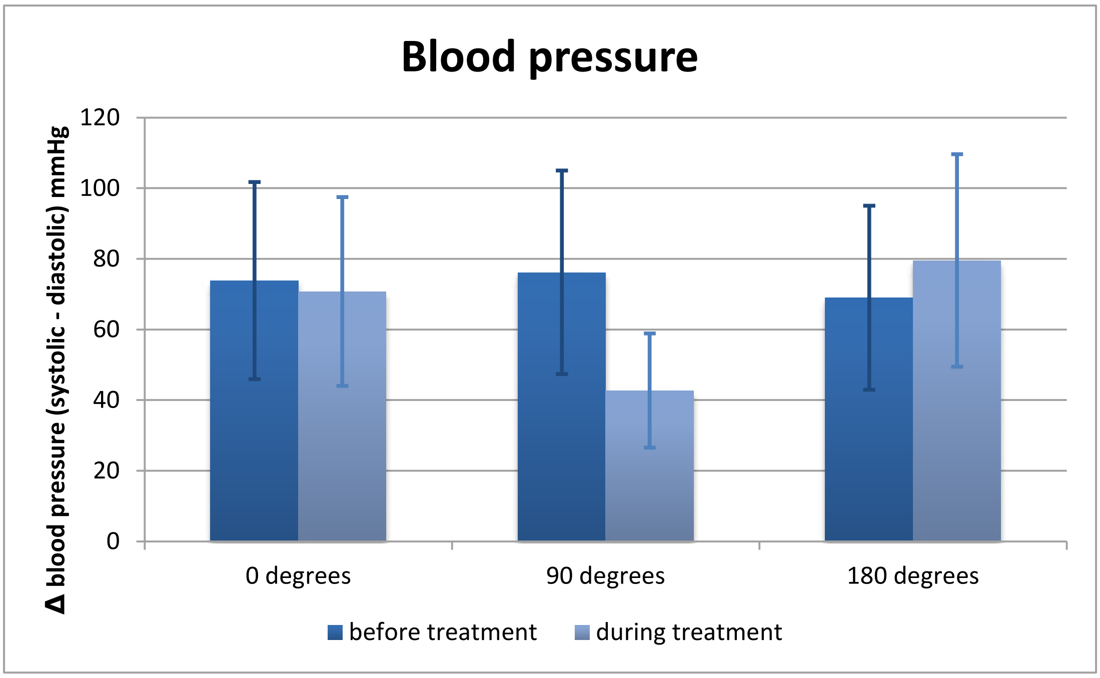


Figure 3.3.5 *Δ blood pressure (systolic minus diastolic blood pressure) before and during treatment*

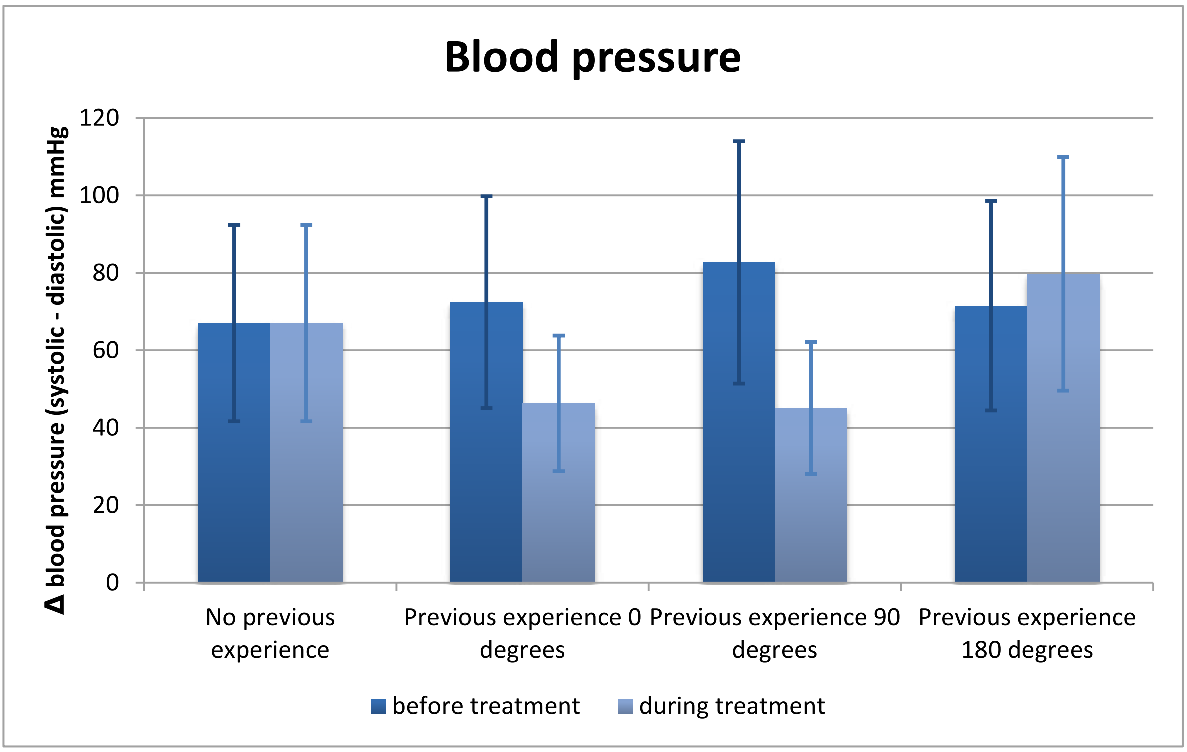


Figure 3.3.6 *Mean difference between systolic and diastolic blood pressure before and during treatment with and without experience in the restraining pen*

Secondly, the effect of experience in the restraining pen was analysed. In figure 3.3.6 it is shown that those animals without experience of the restraining pen had a Δ blood pressure of approximately 67 mmHg, while animals with experience had a slightly larger Δ blood pressure. During treatment however, animals without earlier experience appear to have approximately the same Δ blood pressure, but animals with experience of 0 or 90 degrees rotation tended to display smaller Δ blood

pressure (F=3.12, p=0.07). Animals with experience of being in a restraining pen displayed larger Δ blood pressure.

3.4 Behaviour after restraining and rotation

Laying and rumination were studied in detail to establish treatment affects treatment effects on normal behaviour patterns in the "home" environment.

3.4.1 Rumination activity

On average individuals receiving a treatment with 0 degrees rotation, would start to ruminate approximately 196 (±88) minutes after being returned to their "home" environment. Individuals that experienced a 90 degree rotation started to ruminate after 176 (±79) minutes and those after a 180 degree rotation started to ruminate after 274 (±123) minutes (figure 3.4.1). There were no significant differences between treatments or in relation to previous experiences .
The first rumination bout lasted between 20 and 25 minutes and no significant differences were observed between treatments nor for previous experience with restraining and rotation (figure 3.4.2).

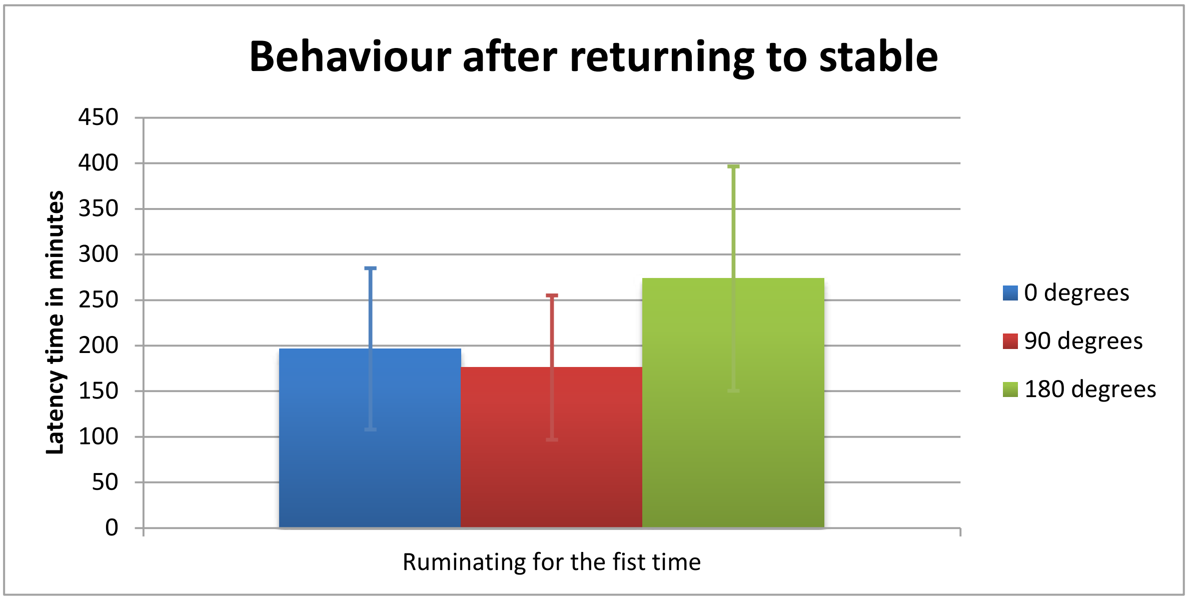


Figure 3.4.1 *Mean latency time to commencement of rumination after return to stable*

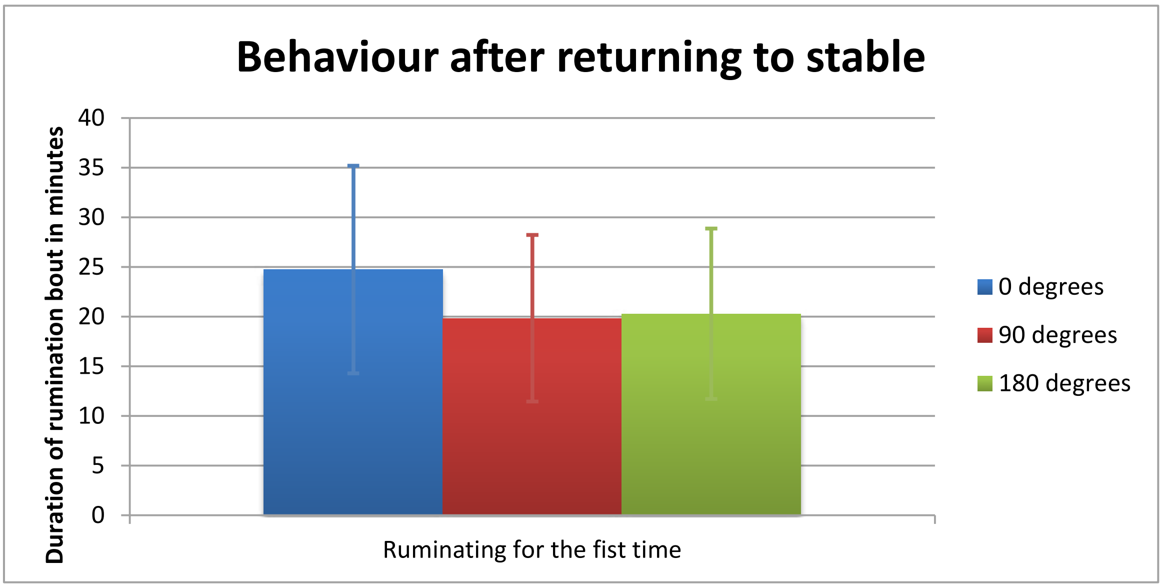


Figure 3.4.2 *Mean duration of first rumination bout after return to stable*

3.4.2 Laying behaviour

On average individuals restrained without rotation, would start their first bout of laying 63 (±26) minutes after being returned to their home environment. After 90 degrees rotation individuals would start the first laying bout after 44 (±18) minutes and after 180 degrees rotation after 72 (±30) minutes (figure 3.4.3). These mean values tended towards significance (F=3.46, p=0.083). There was no significant effect of previous experience of a treatment.
The first laying bout lasted 34, 23 and 38 minutes respectively for the treatments 0, 90 and 180 degrees. There were no significant differences between treatments nor for restraining and rotation experience (figure 3.4.2).

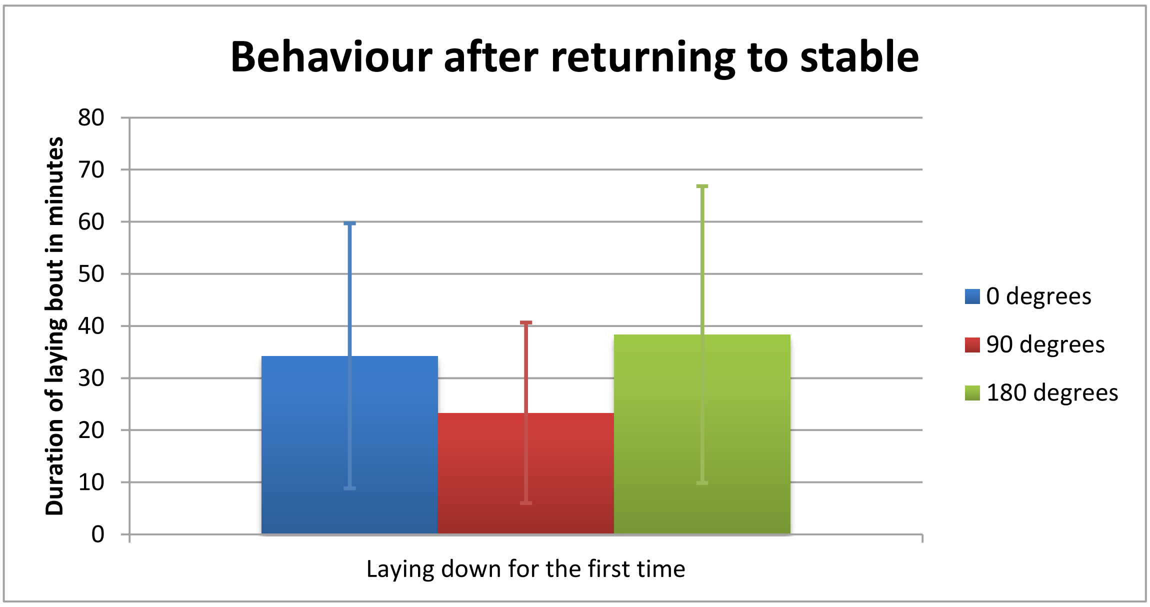


Figure 3.4.3 *Mean latency time to commencement of laying down after return to stable*

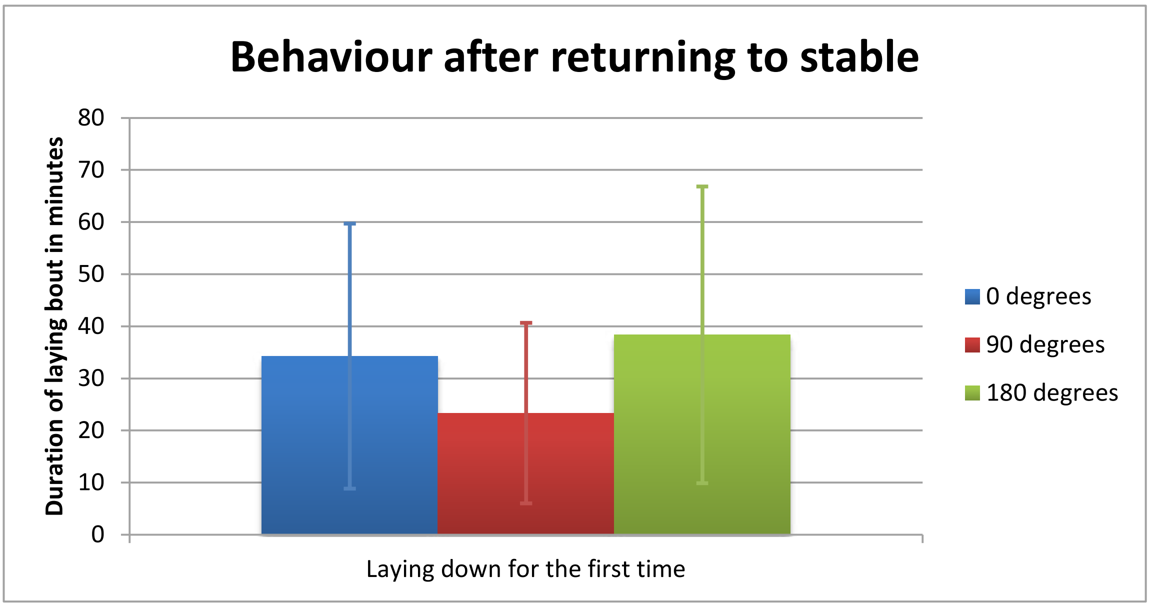


Figure 3.4.4 *Mean duration of first laying down bout after return to stable*

4 Discussion

4.1 Effect of previous handling experiences

The current study was designed to collect the most relevant information for assessing animal welfare during slaughter without stunning using a minimum number of animals. Nine animals were exposed to the same three treatments each, in a randomized order. Although the animals were exposed to the different treatments in randomized order it was not foreseen whether or not experience of the individual would affect the outcome on a following treatment. In the statistical analyses treatment effect was assessed for a so called ‘carry over effect’ from a previous treatment. When necessary the outcomes were corrected for this ‘carry over effect’. One of the outcomes of the study is that previous experiences do effect the behavioural and physiological responses of the animals.

In particular this ‘carry over effect’ was shown when driving the animals in the stable and into the race towards the restraining and rotation pen. The amount of encouragement and stimulation required to drive animals with no prior experience of being in the restraining pen out of the starting pen was significantly lower than for animals with prior experience (figure 3.1.1; F=601.4; p<0.001). This may suggest that individuals had developed a certain level of aversion to be driven out of the staring pen. In concordance with these results, the total duration (time required for this phase) was significantly longer for animals with previous experience of restraint (figure 3.1.3, F=5.5, p=0.022). Surprisingly, this aversion was not present when animals had to enter the restraining pen. It is hypothesised that moving cows from their familiar husbandry environment into the novel restraining pen induces neophobia whereas moving cows from one novel pen to another may not be perceived as an additional challenge.

4.2 Behavioural responses during rotation

Several animals had to be released earlier than the planned period of rotation (3 minutes). In the second week, rotation was aborted (n=3 out of nine occasions) and did not start in 1 occasion due to malfunction of the rotation device. Reasons for this were that in 1 occasions the animals was short of breath followed by hyperventilation and collapsed and a second animal just started to lay down during the standing restraining period.
In the third week the rotation phase was aborted twice (out of 9) because of animals laying down immediate after entering the rotation pen. The animals that collapsed or were laying down in the rotation pen refused to stand up without stimulation. It can be argued that such behaviour is similar to learned helplessness as with a downer cow, where animals are no longer able to cope (Maier et al., 1976).

Behavioural indicators such as defecating, urinating, vocalisations did not seem to show a large variation between individuals (see attachment 1), and may therefore be considered as less valid to assess welfare during restraining.

The only behavioural indicator that did differ across treatments was the display of the whites of the eyes. Whites of the eyes is seen as an indicator of frustration (Sandem et al., 2006) and it is suggested that it may therefore also have indicated fear or stress in the current experiment. The percentage of animals showing white of the eye was greater when animals were rotated (90 degrees or 180 degrees) compared to not being rotated (F=4.11, p=0.016). It is therefore hypothesised that these animals were more fearful when being restrained and rotated than when they were not rotated.

4.3 Effect of rotation on physiological parameters

Implanted telemetry transmitters (Data Science International, DSI®, St Paul, MN) were used to record blood pressure and cardiac activity (ECG) of each individual cow during the experimental period.

4.3.1 Cardiac activity

As clearly shown in figure 3.2.1, the heart rate increases during the first phases of the experiment and decreases after the second phase. The first and second phases involve driving the cow towards another area. It is commonly known that physical activity (locomotion) can increase the individual's mean heart rate (Hopster and Blokhuis, 1994). This increase in heart rate during these locomotion phases was expected. During phase three, the animals were placed in the restraining pen (without being restrained) and heart rate dropped to approximately 100 beats per minute. but did not return to baseline levels (75-80 beats per minute, phase 0, in the stable). This can be explained by the fact that more time was required for the heart rate to return to baseline levels. However a more plausible explanation, is that the emotional reaction to this new environment caused the mean heart rate to remain above the base level (Hopster and Blokhuis, 1994).
From the moment treatment started (phase 5) the pattern of mean heart rate differed significantly between treatments ($F=2.85$, $p<0.001$). It became clear that rotated animals displayed a further decrease in mean heart rate compared to animals that were not rotated. Reasoning behind these findings can be numerous. For example, it can be speculated that animals that were no longer feeling the ground under their hooves (due to gravity) and consequently developed an inactive coping strategy. This would resemble a similar coping strategy as seen with piglets during the 'back-test' (Spake et al., 2012) and a coping strategy seen in fowl described as 'tonic immobility' (Gallup, 1974). This hypothesis is supported by the fact that during rotation animals did not move their legs, while this did occur in all treatments without rotation. However, it is important to state that this drop in the heart rate appears at least after 60 seconds from the beginning of the rotation. In practice, at this point, the sticking should already have taken place. It would be interested to do a further analysis to study if the decrease within the first 60 seconds was significant between treatments.

To study treatment effect on cardiac activity, the mean heart rate before treatment was compared with the mean heart rate after treatment (rotation). No significant effect of treatment on the mean heart rate was observed. Moreover, there was no significant effect of a previous experience in the rotation pen on the mean heart rate.

4.3.2 Blood pressure

Diastolic blood pressure and the difference between systolic and diastolic blood pressure (Δ blood pressure) were used to determine differences in physiological stress related response to the 3 treatments. The means of lowest systolic and diastolic values that have been measured by others were a pressure of 160/110 mmHg (e.g. Doyle et al., 1960).

Initial diastolic and systolic pressure are relative high at the start of the experimental period. In phase 0 (starting pen), the average systolic blood pressure was 188 mmHg and the diastolic blood pressure was 128 mmHg. The high initial blood pressure is most likely induced by the handling and separation of the animals from the group short before the actual experiment. What became clear from this experiment was that while the level of the diastolic blood pressure was not affected by the treatment; meaning that rotation did not caused lower or higher diastolic blood pressure levels, there was an effect of a previous treatment. Animals with a previous experience in the restraining pen showed a lower diastolic blood pressure; and animals that had experienced the 0 degrees and the 90 degrees rotation, this lower diastolic blood pressure was most pronounced.

As another measure for physiological stress, the difference between systolic and diastolic blood pressure, the delta (Δ) was analysed. From these results we can conclude that this difference (Δ) is most, and significantly, affected by treatment. The animals with a 90 degrees treatment showed a much smaller difference between systolic and diastolic blood pressure, compared to the animals receiving the other two treatments. Since the diastolic blood pressure did not significantly drop for this particular treatment, it can be concluded that the smaller Δ blood pressure (systolic-diastolic blood pressure) can only have been caused by a significant drop in the systolic blood pressure. The biological reason for this needs further investigation.

4.4 Behavioural indicators for recovery

In order to test for treatment effects on the recovery to normal behaviour patterns in the "home" environment, laying behaviour and rumination were studied in more detail. Based on the data of laying behaviour and rumination activity there were no significant treatment effects, implying that the treatments did not result in different behaviour recovery patterns. However, there was a trend in latency time for laying down. This was shortest after the 90 degrees rotation treatment.
Because all animals returned to the stable environment at the same time (after all animals had completed their treatment), laying and rumination activity in the waiting area were not taken into account in this current analysis. When studying individual behaviour patterns it should be noted that both laying and rumination did occur in the waiting area after treatment. Therefore, the parameter latency time and duration of laying and rumination from the moment the animals returned to the stable environment, might not have been the most valid ones for assessing recovery. Further analysis is needed to identify differences between treatments especially in the waiting area.

5 Conclusion

Summarizing, the results of the current study show that restraining and rotation is stressful for cows. It has been shown that there is an acute stress effect on being rotated, and it is shown that repeated restraining and rotating is more stressful for the animal. Therefore it can be argued that the negative experience is retained.

However, although distinct parameters such as white of the eyes or blood pressure, do show differences between treatments, there is no overall indication that one treatment (upright, 90 degrees or 180 degrees) is more stressful than the other. Further analysis on, for example heart rate variability, on these data is warranted.

Furthermore, this study has provided some other interesting findings that may be valuable for future studies and/or more in depth analysis of the current data. It was for example found that the refusal to move while being handled and driven from one area to the other is a possible indicator of aversion based on previous experiences. This is an important finding for management procedures in which animals are confronted with negative experiences. The interesting finding so far was that there seemed to be a 'remember factor' in the response on the stressor measures in blood pressure, while this was absent in the mean heart rate. Further analysis is warranted to also include the data of heart rate variability on the restraining.

6 Acknowledgements

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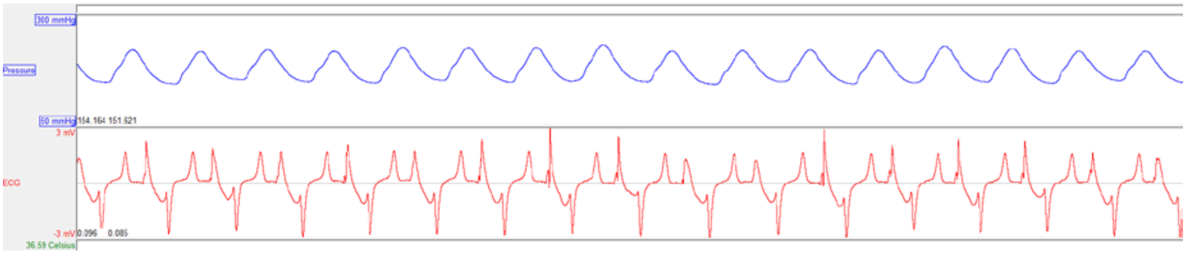
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Appendix 1 Cow nr, treatment and observations

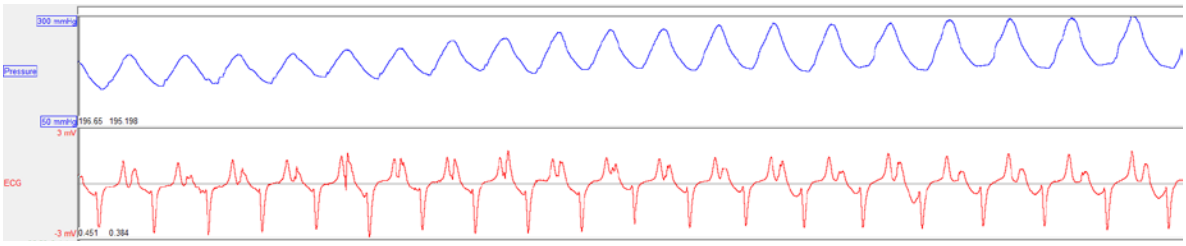
Cow nr	Rotation	PH1 duration	PH1 stimulation	PH1 use of aids	PH1 turning	PH 1 defecating	PH1 urinating	PH2 duration	PH2 stimulation	PH2 use of aids	PH2 turning	PH2 defecating	PH2 urinating	PH3 posture	PH3 vocalisation	PH3 resistance	PH3 defecating	PH3 urinating	PH5 posture	PH5 vocalisation	PH5 resistance	PH5 defecating	PH5 urinating	PH5 white of the eye	PH7 leaving
5778	0	44	1	1	1	0	0	386	3	3	1	1	0	1	1	2	0	0	1	1	2	0	1	2	2
en 4903	180	11	2	2	1	0	0	6	2	2	1	0	0	1	1	1	0	1	1	1	2	0	0	3	1
5611	90	11	1	1	1	0	0	190	2	1	1	0	0	1	1	1	0	0	1	1	2	0	0	3	1
5754	90	20	1	1	1	0	0	14	2	2	1	0	0	1	1	1	0	0	1	1	3	0	0	2	3
5187	0	12	1	2	1	0	0	9	1	1	1	0	0	1	1	1	0	0	1	1	2	0	0	1	1
3672	180	12	1	1	1	0	0	7	1	1	1	0	0	1	1	2	0	1	1	1	2	1	0	3	3
5864	180	10	1	1	1	0	0	10	1	2	1	0	0	1	1	2	0	0	1	3	2	0	0	1	3
5197	0	7	1	1	1	0	0	6	1	1	1	0	0	1	1	1	0	0	1	3	2	0	0	1	2
6066	90	9	1	1	1	0	0	10	1	1	1	0	0	1	1	1	0	0	1	3	2	0	1	3	3
5778	90	176	3	2	2	0	0	17	3	2	1	0	0	1	1	1	2	0	1	3	1	0	0	4	
4903	0	17	2	2	1	0	0	8	2	2	1	0	0	1	1	1	0	0	1	1	2	0	0	2	
5611	180	92	3	3	3	0	0	487	2	3	2	1	0	1	1	1	0	0	1	2	1	0	0	3	
5754	0	11	2	1	1	0	0	10	2	2	1	0	0	1	1	2	0	0	1	2	2	0	0	2	
5187	180	16	1	1	1	0	0	13	1	1	1	0	0	1	1	1	0	0	1	2	3	0	0	3	
3672	90	21	2	2	2	0	0	17	2	2	1	0	0	1	1	1	0	1	2	1	3	2	1	4	
5864	0	10	1	1	1	0	0	10	2	2	1	0	0	1	1	2	0	0	2	2	2	0	0	2	3
5197	90	9	1	1	1	0	0	13	1	2	1	0	0	1	1	1	0	0							2
6066	180	12	1	1	1	0	0	6	1	2	1	0	0	1	2	2	0	1	1	2	3	0	0	2	2
5778	180	92	3	2	2	0	0	4	2	2	1	0	0	1	1	1	0	0	1	2	3	1	0	3	
4903	90	210	3	2	3	0	0	7	3	2	1	0	0	1	1	1	0	0	1	1	1	0	1	4	1
5611	0	27	2	3	1	0	0	278	3	3	3	0	0	1	1	2	1	0	2	1	2	1	1	4	1
5754	180	188	3	3	3	0	0	11	2	3	1	0	0	1	1	2	0	0	1	2	1	0	0	3	
5187	90	15	2	2	2	0	0	10	1	2	1	0	0	1	1	1	0	0	1	2	1	0	0	3	1
3672	0	39	1	2	1	0	0	9	1	2	1	0	0	2	1	1	0	0							2
5864	90	38	2	2	2	1	0	10	3	3	1	0	0	1	1	2	0	0							2
5197	180	7	1	1	1	0	0	4	1	1	1	0	0	1	1	1	1	1	1	2	2	0	0	3	1
6066	0	5	1	1	1	0	0	11	1	2	1	0	0	1	2	2	0	0	1	2	2	0	0	1	1

Appendix 2 Illustration of blood pressure and ECG of an animal rotated 180 degrees

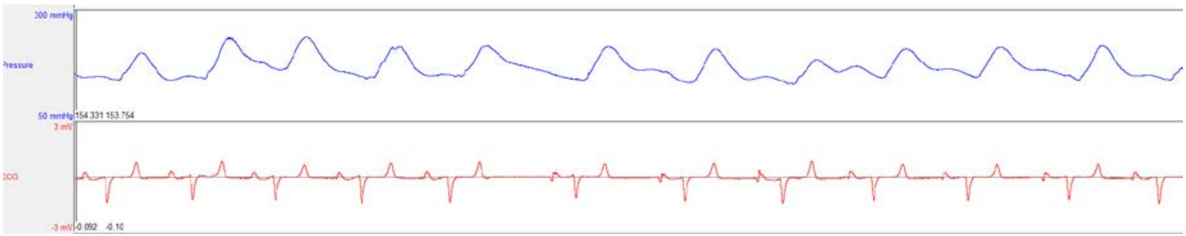
Phase 4 (just before rotating)



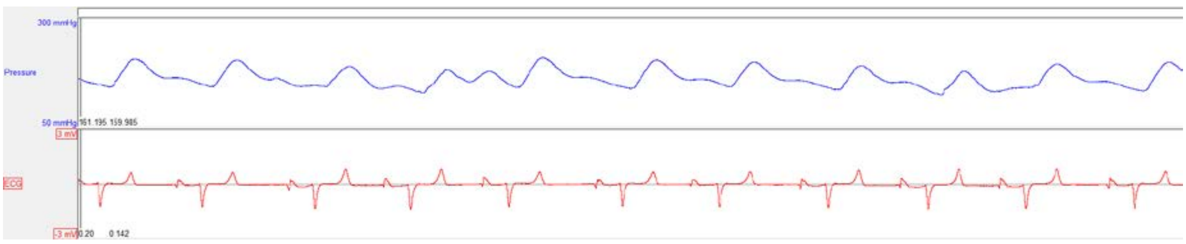
Phase 5 0-30 sec



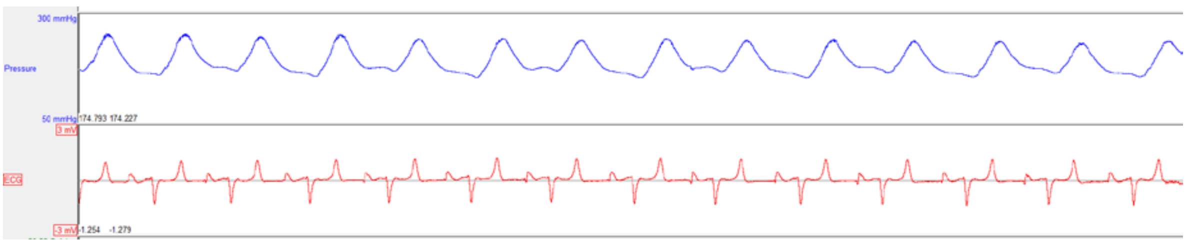
Phase 5 90-120 sec



Phase 5 150-180 sec



Just before releasing



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